

## Evaluation of the antimalarial and antileishmanial activity of plants from the Greek island of Crete

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Received: 9 March 2006 / Accepted: 19 May 2006 / Published online: 1 September 2006  
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**Abstract** Different parts of 65 plant species from the Greek island of Crete have been extracted and the 249 extracts obtained have been investigated for in-vitro antiprotozoal activity. Their activity against chloroquine-sensitive (D6) and resistant (W2) strains of *Plasmodium falciparum* and *Leishmania donovani* promastigotes was determined. Their cytotoxicity on a mammalian kidney fibroblast (Vero) cell line was also tested. Dichloromethane and methanol extracts of *Berberis cretica* and methanol extracts of *Cytinus hypocistis* subsp. *hypocistis*, *C. hypocistis* subsp. *orientalis*, and *C. ruber* had significant activity against both strains of *P. falciparum* ( $IC_{50} < 10 \mu\text{g mL}^{-1}$ ). Dichloromethane extracts of *Eryngium ternatum*, *Origanum dictamnus*, and *Origanum microphyllum*, and the methanolic extract of *Eryngium amarginum* had significant activity against *Leishmania donovani* ( $IC_{50} < 10 \mu\text{g mL}^{-1}$ ). None of the extracts was cytotoxic.

**Keywords** Screening · Antimalarial activity · Antileishmanial activity · Greece · Crete

### Introduction

Malaria and leishmaniasis are two of the most common parasitic diseases and infect a large human population over five continents. Malaria is a public health problem in over 100 countries worldwide, inhabited by approximately 40% of the world's population, i.e. over 2 billion people. The incidence of malaria in the world has been estimated to be approximately 300 million clinical cases each year. Countries in tropical Africa account for more than 90% of these cases, with a vast number of deaths occurring among young children. Malaria mortality is estimated at almost 1 million deaths worldwide per year. Although malaria has been widely eradicated in many parts of the world, the global number of cases continues to increase in continents where malaria is still present. One of the reasons for this alarming situation is the rapid increase in resistance of malaria parasites to antimalarial drugs, especially to chloroquine, one of the most frequently prescribed drugs [1].

Another important parasitosis is leishmaniasis, a disease caused by 20 different species of human pathogen all of which belong to the genus *Leishmania*. Leishmaniasis is a parasitic disease with a wide range of clinical symptoms, from self-healing ulcers, cutaneous leishmaniasis (CL), to progressive nasopharyngeal infections (mucocutaneous leishmaniasis, MCL) and disseminating visceral leishmaniasis (VL), which can be fatal if left untreated. According to the WHO there are approximately 12 million cases of leishmaniasis globally, with 2 million new cases of CL and half a million new cases of VL per year [2, 3]. Visceral leishmaniasis also occurs as an opportunistic infection in HIV-infected patients [4].

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Most of the treatments available for parasitic diseases are 20 or more years old and suffer from limited efficacy and frequent undesirable side effects. In addition, the vast majority of people affected by such diseases do not have the financial resources to cover the costs of full treatment. For these reasons one of the major goals of the scientific community has been the discovery of new effective and accessible drugs, particularly from traditional medicinal plant sources.

Plant-derived natural products could be a promising source of bioactive compounds including antiparasitic drugs. The biodiversity of plants on earth, according to a recently published research report in *Nature* [5], is concentrated in 25 “hot spots”. One of these spots is the Mediterranean basin. Greece, with more than 5,700 plant species (among them 740 endemic), is an especially large potential reservoir of unique chemical structures with possible biological activity [6].

Crete, the southern-most island of Greece, has a unique flora, with more than approximately 1,800 plant species; among these approximately 180 are endemic to Crete [7]. The number of taxa per 1,000 km<sup>2</sup> is 219; the European average is 11.9 and the total for Greece is 43.2. The number of endemic taxa per 1,000 km<sup>2</sup> is, moreover, 22, in contrast with the average of 0.33 for Europe and the 7.2 for Greece. Many of the endemic taxa of Crete are narrow-endemics, and 42% of the plant taxa found above 1,500 m (mountain flora) are endemic to Greece. The island therefore has the richest mountain flora of Greece and the most diverse flora of the Mediterranean basin [8].

In our attempt to find new plants with activity against parasitic protozoa we report results from *in vitro* evaluation of 249 crude extracts of plants from the Greek island of Crete against both malaria and leishmania parasites. Some plants extracts with promising antimarial and antileishmanial activity were identified.

## Material and methods

### Plant material and preparation of extracts

All plant material except *Echinops ritro* and *Bryonia cretica* subsp. *dioica* was collected in Crete between 1997 and 2001 and was identified by Dr E. Kalpoutzakis. Specimens are kept in the herbarium of Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Greece. Botanical names, plant parts, voucher specimen number, and place and date of collection are listed in Table 1. The names of the plants are those

given by Turland et al. [9] except *Astragalus creticus* subsp. *creticus* and the members of the genus *Cytinus*, which are named in accordance with the Flora Europaea [10, 11].

Different parts (as specified) of the plant material were reduced to small pieces, dried at room temperature, and powdered in a grinder. The powdered material was then extracted solvents of increasing polarity—dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>, DCM), methanol, and, finally, water. The extraction procedure was repeated three times for each solvent. The organic solvent was removed by vacuum distillation and aqueous extracts were lyophilized. All residues were then stored in a dry place protected from light.

### Strains of *Plasmodium falciparum* and *Leishmania donovani*

Strains of *Plasmodium falciparum*, Sierra Leone D6 (chloroquine sensitive) and Indochina W2 (chloroquine resistant) were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Washington DC, USA). The culture of *Leishmania donovani* promastigote (1S2D strain) was provided by Professor R. Balana-Fouce, University of Leon, Spain.

### Assay for antileishmanial activity

Antileishmanial activity was tested *in vitro* on a culture of *Leishmania donovani* promastigotes. In a 96-well microplate assay appropriately diluted extracts were added to the leishmania promastigotes culture ( $2 \times 10^6$  cells mL<sup>-1</sup>). The plates were incubated at 26°C for 72 h and growth of leishmania promastigotes was determined by use of the Alamar blue assay [12]. Pentamidine and amphotericin B were used as the standard antileishmanial agents.

### Assay for antimarial activity

The antimarial activity of the extracts was determined *in vitro* on chloroquine-sensitive and resistant strains of *Plasmodium falciparum* in a 96-well microplate assay. The assay is based on evaluation of the effect of the extracts on the growth of asynchronous cultures of *P. falciparum*, determined by assay of parasite lactate dehydrogenase (pLDH) activity [13]. Test samples diluted appropriately in RPMI medium were added to the cultures of *P. falciparum* (2% hematocrit, 2% parasitemia) in the wells of clear flat-bottomed 96-well plates. The plates were placed in a modular incubation chamber, flushed with a gas mixture of 90%

**Table 1** Plants of the Cretan flora that were investigated

Name	Family	Plant part	Voucher	Origin	Collection date
<i>Anchusa cespitosa</i> Lam. <sup>a</sup>	Boraginaceae	Whole	KL064	West Crete	May 1999
<i>Anthemis rigida</i> (Sm) Boiss. & Heldr. subsp. <i>rigida</i>	Compositae	Whole	KL123	Central Crete	May 2001
<i>Aristolochia reticula</i> Lam. <sup>a</sup>	Aristolochiaceae	Rhizome Flowers Aerial	KL001R KL001 KL001Y	East Crete	April 2000
<i>Arum creticum</i> Boiss. & Heldr.	Araceae	Bulbs Aerial	KL002R KL002Y	Central Crete	April 1998
<i>Arum idaeum</i> Coustr. & Gand. <sup>a</sup>	Araceae	Aerial Bulbs	KL003Y KL003R	West Crete	May 1999
<i>Asphodeline lutea</i> (L.) Rchb.	Liliaceae	Aerial Rhizome	KL065Y KL065R	Central Crete	April 1999
<i>Astragalus angustifolius</i> Lam. subsp. <i>angustifolius</i>	Fabaceae	Aerial	KL067Y	Central Crete	June 1999
<i>Astragalus creticus</i> Lam. subsp. <i>creticus</i>	Leguminosae	Radix Aerial Rhizome	KL067R KL004Y KL004R	Central Crete	May 1999
<i>Atractylis gummifera</i> L.	Compositae	Aerial Resin Rhizome	KL005Y KL005G KL005R	Central Crete	June 1998
<i>Bellis longifolia</i> Boiss. & Heldr. in Boiss. <sup>a</sup>	Compositae	Whole	KL068	West Crete	May 1999
<i>Berberis cretica</i> L.	Berberidaceae	Radix Fruits	KL006R KL006F	Central Crete	August 1999
<i>Bryonia cretica</i> L. subsp. <i>cretica</i>	Cucurbitaceae	Aerial Rhizome	KL006Y KL007Y KL007B	East Crete	April 1998
<i>Bryonia cretica</i> subsp. <i>dioica</i>	Cucurbitaceae	Rhizome	KL116	Peloponnese	April 2000
<i>Campanula tubulosa</i> Lam. <sup>a</sup>	Campanulaceae	Whole	KL008	Central Crete	June 1998
<i>Centaurea idaea</i> Boiss. & Heldr. in Boiss. <sup>a</sup>	Compositae	Aerial	KL009	Central Crete	June 1998
<i>Centaurea raphanina</i> Sm. subsp. <i>araphanina</i> <sup>a</sup>	Compositae	Whole	KL010	Central Crete	April 1998
<i>Cichorium spinosum</i> L.	Compositae	Whole	KL011	Central Crete	April 1997
<i>Cistus salvifolius</i> L.	Cistaceae	Aerial	KL059	Central Crete	June 1997
<i>Cistus creticus</i> L. subsp. <i>creticus</i>	Cistaceae	Aerial Resin	KL057 KL057R	Central Crete	June 1998
<i>Cistus creticus</i> L. subsp. <i>eriocephalus</i>	Cistaceae	Aerial	KL058	Central Crete	May 1997
<i>Cistus monspeliensis</i> L.	Cistaceae	Aerial	KL060	East Crete	June 1999
<i>Cistus parviflorus</i> Lam.	Cistaceae	Aerial	KL012	Central Crete	June 1998
<i>Citrus aurantium</i> L. <sup>b</sup>	Rutaceae	Leaves	KL144	Central Crete	June 1999
<i>Cynoglossum columnae</i> Ten.	Boraginaceae	Aerial	KL013b	Central Crete	May 1999
<i>Cytinus hypocistis</i> (L.) L. subsp. <i>hypocistis</i>	Rafflesiaceae	Whole	KL014	Central Crete	April 1998
<i>Cytinus hypocistis</i> (L.) L. subsp. <i>orientalis</i>	Rafflesiaceae	Whole	KL015	Korinth	April 1998
<i>Cytinus ruber</i> Fritsch.	Rafflesiaceae	Whole	KL016	Central Crete	April 1998
<i>Daphne sericea</i> Vahl	Thymelaeacea	Aerial	KL070	West Crete	April 1999
<i>Echinops ritro</i> L.	Compositae	Aerial Radix	KL017Y KL017R	Central Greece	July 2000
<i>Echinops spinosissimus</i> Turra subsp. <i>spinosissimus</i>	Compositae	Aerial	KL018Y	Central Crete	May 1997
<i>Erodium moschatum</i> (L.) L'Hér.	Geraniaceae	Radix	KL018R	Central Crete	June 1998
<i>Eryngium amorginum</i> Rech. fil. <sup>a</sup>	Apiaceae	Aerial	KL019	Central Crete	March 1998
<i>Eryngium campestre</i> L.	Apiaceae	Aerial	KL100	East Crete	June 2000
<i>Eryngium creticum</i> Lam.	Apiaceae	Aerial	KL107	Central Crete	June 2000
<i>Eryngium maritimum</i> L.	Apiaceae	Aerial	KL202	West Crete	May 2000
<i>Eryngium ternatum</i> Proiret <sup>a</sup>	Apiaceae	Aerial	KL021	West Crete	May 2000
<i>Galium fruticosum</i> Willd. <sup>a</sup>	Rubiaceae	Aerial	KL022	West Crete	May 2000
<i>Helminthotheca echoidea</i> (L.) Holub. [= <i>Picris echoidea</i> L.]	Compositae	Aerial	KL074	West Crete	May 1998
<i>Inula pseudolimonella</i> Rech.f. <sup>a</sup>	Compositae	Aerial	KL031	Central Crete	May 1999
<i>Iris unguicularis</i> Poir. subsp. <i>cretensis</i> (Janka) A.P. Davis & Jury <sup>a</sup>	Iridaceae	Rhizome	KL024	East Crete	April 1997
<i>Lagenaria vulgaris</i> Ser. <sup>b</sup>	Cruciferae	Seeds	KL082	East Crete	October 1999

**Table 1** continued

Name	Family	Plant part	Voucher	Origin	Collection date
<i>Lavandula stoechas</i> L.	Labiatae	Aerial	KL099B	West Crete	May 2001
<i>Leontodon tuberosus</i> L.	Compositae	Whole	KL038	Central Crete	March 1998
<i>Luffa cylindrica</i> Roem. <sup>b</sup>	Cruciferae	Seeds	KL079S	Central Crete	October 1998
<i>Luzia cretica</i> (L.) Greuter & Burdet <sup>a</sup>	Cruciferae	Aerial	KL072	East Crete	April 1999
<i>Nepeta melissifolia</i> Lam. <sup>a</sup>	Labiatae	Aerial	KL103	East Crete	May 2000
<i>Onosma erecta</i> Sm. subsp. <i>erecta</i> <sup>a</sup>	Boraginaceae	Aerial	KL025	West Crete	May 1999
<i>Origanum dictamnus</i> L. <sup>a</sup>	Labiatae	Aerial	KL026	Central Crete	August 1997
<i>Origanum microphyllum</i> (Benth.) Vogel <sup>a</sup>	Labiatae	Aerial	KL078	East Crete	June 2001
<i>Parietaria cretica</i> L.	Urticaceae	Aerial	KL027	West Crete	April 1999
<i>Petromarula pinnata</i> (L.) A.DC. <sup>a</sup>	Campanulaceae	Aerial	KL028	Central Crete	April 1998
<i>Phlomis cretica</i> C. Presl in J. & C. Presl <sup>a</sup>	Labiatae	Aerial	KL029	Central Crete	April 1998
<i>Phlomis lanata</i> Wild. <sup>a</sup>	Labiatae	Aerial	KL030	Central Crete	April 1997
<i>Ptilostemon chamaepeuce</i> (L.) Less.	Compositae	Aerial	NEK009	West Crete	May 1999
<i>Rhamnus lycioides</i> L. subsp. <i>oleoides</i> (L.) Jahand & Maire	Rhamnaceae	Aerial	KL032	Central Crete	June 1998
<i>Sarcopoterium spinosum</i> (L.) Spach	Rosaceae	Aerial	KL033	Central Crete	April 1998
<i>Sideritis syriaca</i> L. subsp. <i>syriaca</i> <sup>a</sup>	Labiatae	Flowering stems	KL035	Central Crete	July 1998
<i>Spinacia oleracea</i> L. <sup>b</sup>	Chenopodiaceae	Aerial	KL143		
<i>Stachys spinosa</i> L. <sup>a</sup>	Labiatae	Aerial	KL036	Central Crete	June 1998
<i>Staelhelina petiolata</i> (L.) Hilliard & B.L. Burtt <sup>a</sup>	Compositae	Aerial	KL073	Central Crete	May 1999
<i>Styrax officinalis</i> L.	Styracaceae	Stems Flowers	KL037K KL037F	Central Crete	April 1998
<i>Tordylium apulum</i> (L.)	Apiaceae	Rosette Aerial	KL039R KL039	Central Crete	April 1998
<i>Verbascum arcturus</i> (L.) <sup>a</sup>	Scrophulariaceae	Aerial (annual) Aerial (perennial)	KL040Y KL040P	West Crete	April 1999
<i>Verbascum spinosum</i> L. <sup>a</sup>	Scrophulariaceae	Aerial	KL048	West Crete	June 1997
<i>Viscum album</i> L.	Loranthaceae	Aerial	KL061	East Crete	September 2000

<sup>a</sup>Endemic plants of Greece<sup>b</sup>Species introduced

$\text{N}_2$ , 5%  $\text{CO}_2$  and 5%  $\text{O}_2$ , and incubated at 37°C for 72 h. Growth of the parasite in each well was determined by pLDH assay using Malstat reagent (Flow, Portland, OR, USA) as described elsewhere [14]. Standard antimalarial agents chloroquine and artemisinin were included as positive controls and DMSO was used as vehicle control.

#### Assay for cytotoxicity

Plant extracts were also tested for cytotoxicity in a culture of Vero cells (monkey kidney fibroblast, obtained from ATCC) up to a highest concentration of 47.6  $\mu\text{g mL}^{-1}$ , the highest concentration used in the antimalarial assays. The assay was performed in 96-well tissue culture-treated microplates, as described elsewhere [15]. Briefly, the wells of the plate were seeded with 25,000 cells per well and incubated for 24 h. Diluted samples were added and plates were again incubated for 48 h. The number of viable cells was determined by use of a modified version of the neutral red assay procedure [16]. IC<sub>50</sub> values were determined from logarithmic graphs of growth inhibi-

tion against concentration. Doxorubicin was used as a positive control ( $\text{IC}_{50}=5 \mu\text{g mL}^{-1}$ ) and DMSO was used as vehicle control. If there was no effect of the test material at the highest concentration (47.6  $\mu\text{g mL}^{-1}$ ) the material was regarded as not cytotoxic (NT).

#### Results and discussion

With the objective of identifying bioactive extracts against malaria and leishmania 65 plant species of the Greek flora were investigated (Table 1). Aerial parts, whole plants, roots, radix, seeds, fruits, flowers, rosettes, flowering stems, and leaves were extracted to afford 249 extracts. Stock solutions of the dried extracts were prepared in DMSO at a concentration of 20 mg  $\text{mL}^{-1}$ .

The extracts, at three concentrations, 4, 20, and 100  $\mu\text{g mL}^{-1}$ , were tested against promastigote cultures of *L. donovani* and IC<sub>50</sub> and IC<sub>90</sub> values (the concentrations causing 50 and 90% cell deaths, respectively) were calculated. Sixty-five extracts had antileishmanial activity (Table 2). Of these, forty-three were active

with  $IC_{50}$  and  $IC_{90}$  values below 50 and 100  $\mu\text{g mL}^{-1}$ , respectively. The four most potent extracts, with  $IC_{50}$  values below 10  $\mu\text{g mL}^{-1}$ , were those from *Eryngium amorginum*, *E. ternatum*, *Origanum dictamnus*, and *O. microphyllum*. Their  $IC_{90}$  values ranged from 8 to 19  $\mu\text{g mL}^{-1}$  (Table 2).

Plant extracts were also tested for antimalarial activity up to a highest concentration of 47.6  $\mu\text{g mL}^{-1}$ .  $IC_{50}$  values (concentrations causing 50% inhibition of growth) were obtained from dose-response plots. Twenty-two extracts had activity against at least one of the strains of *P. falciparum*, with  $IC_{50}$  values ranging from 2.6 to 46  $\mu\text{g mL}^{-1}$ . Seven extracts had promising antimalarial activity against both strains, with  $IC_{50}$  values <10  $\mu\text{g mL}^{-1}$ . Of these, two were methanolic and aqueous extracts of *Berberis cretica* ( $IC_{50}$  2.6 and 5.8  $\mu\text{g mL}^{-1}$ , respectively) and five were MeOH and H<sub>2</sub>O extracts of *Cytinus* species ( $IC_{50}$  2.8–9.0  $\mu\text{g mL}^{-1}$ ).

None of the extracts was cytotoxic to mammalian cells up to the highest concentration of 47.6  $\mu\text{g mL}^{-1}$ . Plant extracts with promising activity against the malaria and/or leishmania parasites were investigated in more detail.

#### *Berberis cretica*

The aerial parts, the radix, and the fruits of *B. cretica* were investigated. The MeOH and H<sub>2</sub>O extracts of the radix had significant activity against both chloroquine-sensitive and resistant *P. falciparum* strains with  $IC_{50}$  values of 2.6 and 5.8  $\mu\text{g mL}^{-1}$ , respectively, against the D6 strain and 6.2 and 8.7  $\mu\text{g mL}^{-1}$ , respectively, against the W2 strain. The antimalarial activity of these extracts might be attributed mainly to isoquinoline alkaloids, for example berberin, which have been isolated from the radix [17] and are known to have antimalarial activity [18]. The MeOH and H<sub>2</sub>O extracts of the radix, the DCM and MeOH extracts of the aerial parts, and the DCM extract of the fruits had moderate activity against *L. donovani*. None had cytotoxic activity in our bioassay.

#### *Cytinus hypocistis* subsp. *hypocistis*, *C. hypocistis* subsp. *orientalis*, and *C. ruber*

The MeOH and the H<sub>2</sub>O extracts of the whole plant of the three *Cytinus* species were investigated and found to be active against *P. falciparum*. The  $IC_{50}$  values for *C. hypocistis* subsp. *hypocistis* were, respectively, 2.8 and 10  $\mu\text{g mL}^{-1}$  against the D6 strain and 1.5 and 7.5  $\mu\text{g mL}^{-1}$  against the W2 strain, for *C. hypocistis* subsp. *orientalis* were 6.2 and 3.8  $\mu\text{g mL}^{-1}$  against D6 and 6.0 and 2.2  $\mu\text{g mL}^{-1}$  against W2, and, finally, for

*C. ruber* were 9.0 and 12  $\mu\text{g mL}^{-1}$  against D6 and 9.8 and 9.8  $\mu\text{g mL}^{-1}$  against W2. Previous studies of *Cytinus* species from Greece [19] have revealed the presence of several hydrolyzable tannins, a group of compounds previously reported to have antimalarial activity [20].

#### *Origanum dictamnus* and *O. microphyllum*

The DCM extract of the aerial parts of both *Origanum* species had significant activity against *L. donovani* with  $IC_{50}$  values of 9.2 and 8.8  $\mu\text{g mL}^{-1}$ , respectively, and  $IC_{90}$  values of 18 and 17  $\mu\text{g mL}^{-1}$ . *Origanum* species are traditionally used as herbs in the Mediterranean diet, especially in Crete [21]. *Origanum dictamnus* is widely cultivated in east Crete. The volatile constituents of both species have recently been investigated [22, 23]. The activity of the extracts can be attributed to non-polar compounds of the dichloromethane extract, for example triterpenes, ursolic acid, and oleanolic acid, previously identified in Greek *Labiateae* [24] and recently shown to have strong activity against *Leishmania* sp. [25]. The same extracts have only moderate activity against *P. falciparum*, and no cytotoxicity, indicating their selective activity against leishmania cells.

#### *Eryngium amarginum* and *E. ternatum*

Five representatives of *Eryngium* sp. were investigated. The MeOH extract of *E. amarginum* and the DCM extract of *E. ternatum* had the most interesting activity against *L. donovani*;  $IC_{50}$  values were 2.0 and 9.5  $\mu\text{g mL}^{-1}$ , respectively. Coumarins and flavonoids have previously been isolated from *E. campestre*, [26, 27]. Flavonoids and saponins, compounds previously shown to have activity against *Leishmania* [28, 29], have been isolated from *E. maritimum* [30, 31]. *E. amarginum*, the species affording the most potent extracts, is an endemic plant of Greece that has not yet been studied detail. It is a good candidate for providing bioactive compounds. The same extracts had no significant antimalarial activity or cytotoxicity.

Our results lead to the conclusion that the extended biodiversity of the Cretan flora could lead to the discovery of new antiprotozoal agents. The fact that more than 40% of the best selling pharmaceuticals in use today are derived from natural products [32] and the results of this survey must further encourage investigation of plant extracts for efficient treatment of malaria and leishmania. Future perspectives include identification of the bioactive compounds by bioassay-guided fractionation of the extracts and study of their mode of action.

**Table 2** The activity of the bioactive plant extracts against *Leishmania donovani* and *Plasmodium falciparum*

Plant	Plant part and extract	<i>L. donovani</i>		<i>P. falciparum</i>		Cytotoxicity
		IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	IC <sub>90</sub> ( $\mu\text{g mL}^{-1}$ )	IC <sub>50</sub> D6 ( $\mu\text{g mL}^{-1}$ )	IC <sub>50</sub> W2 ( $\mu\text{g mL}^{-1}$ )	
<i>Anchusa cespitosa</i>	Whole/DCM	58	> 100	30	9.5	NC
<i>Aristolochia reticata</i>	Aerial/DCM	37	82	NA	NA	NC
<i>Arum creticum</i>	Aerial/DCM	45	86	NA	NA	NC
	Bulbs/H <sub>2</sub> O	21	80	NA	NA	NC
<i>Arum idaeum</i>	Aerial/MeOH	44	95	NA	NA	NC
<i>Asphodeline lutea</i>	Radix/DCM	43	84	30	18	NC
<i>Atractylis gummifera</i>	Aerial/DCM	46	87	NA	NA	NC
<i>Bellis longifolia</i>	Whole/DCM	54	> 100	NA	NA	NC
	Whole/MeOH	45	87	NA	NA	NC
<i>Berberis cretica</i>	Radix/MeOH	40	84	2.6	6.2	NC
	Radix/H <sub>2</sub> O	68	> 100	5.8	8.7	NC
	Aerial/DCM	70	> 100	NA	NA	NC
	Aerial/MeOH	49	> 100	28	37	NC
	Fruits/DCM	44	88	NA	NA	NC
<i>Bryonia cretica</i> subsp. <i>cretica</i>	Aerial/DCM	52	> 100	NA	NA	NC
<i>Campanula tubulosa</i>	Whole/DCM	48	98	NA	NA	NC
<i>Centaurea idaea</i>	Aerial/DCM	47	100	NA	NA	NC
<i>Centaurea raphanina</i> subsp. <i>raphanina</i>	Whole/DCM	49	> 100	NA	NA	NC
<i>Cichorium spinosum</i>	Whole/DCM	94	> 100	NA	NA	NC
<i>Cistus salvifolius</i>	Aerial/DCM	51	> 100	NA	NA	NC
<i>Cistus creticus</i> subsp. <i>creticus</i>	Aerial/DCM	56	> 100	NA	NA	NC
<i>Cistus creticus</i> subsp. <i>creticus</i>	Resin/DCM	43	86	46	NA	NC
<i>Cistus creticus</i> subsp. <i>eriocephalus</i>	Aerial/DCM	47	100	NA	NA	NC
	Aerial/MeOH	68	> 100	NA	NA	NC
<i>Cistus monspeliensis</i>	Aerial/DCM	47	93	NA	NA	NC
<i>Cistus parviflorus</i>	Aerial/DCM	54	> 100	NA	NA	NC
<i>Citrus aurantium</i>	Leaves/DCM	13	58	NA	NA	NC
<i>Cynoglossum columnae</i>	Aerial/DCM	100	> 100	NA	NA	NC
<i>Cytinus hypocistis</i> subsp. <i>hypocistis</i>	Whole/MeOH	NA	NA	2.8	1.5	NC
	Whole/H <sub>2</sub> O	NA	NA	10	7.5	NC
<i>Cytinus hypocistis</i> subsp. <i>orientalis</i>	Whole/MeOH	NA	NA	6.2	6	NC
	Whole/H <sub>2</sub> O	NA	NA	3.8	2.2	NC
<i>Cytinus ruber</i>	Whole/MeOH	NA	NA	9	9.8	NC
	Whole/H <sub>2</sub> O	NA	NA	12	9.8	NC
<i>Daphne sericea</i>	Aerial/DCM	100	> 100	NA	NA	NC
<i>Echinops ritro</i>	Aerial/DCM	74	> 100	NA	NA	NC
	Radix/DCM	45	89	NA	NA	NC
<i>Echinops spinosissimus</i> subsp. <i>spinosissimus</i>	Aerial/DCM	84	> 100	NA	NA	NC
	Radix/DCM	48	90	NA	NA	NC
<i>Erodium moschatum</i>	Aerial/DCM	40	93	NA	NA	NC
<i>Eryngium amarginatum</i>	Aerial/DCM	46	87	NA	NA	NC
	Aerial/MeOH	2	8	32	25	NC
<i>Eryngium campestre</i>	Aerial/DCM	36	83	NA	NA	NC
	Aerial/MeOH	15	74	NA	NA	NC
<i>Eryngium creticum</i>	Aerial/DCM	38	83	NA	NA	NC
	Aerial/MeOH	35	83	NA	NA	NC
<i>Eryngium maritimum</i>	Aerial/DCM	32	80	30	27	NC
<i>Eryngium ternatum</i>	Aerial/DCM	9.5	19	36	32	NC
	Aerial/MeOH	21	80	NA	NA	NC
<i>Galium fruticosum</i>	Aerial/DCM	44	86	NA	NA	NC
<i>Galium fruticosum</i>	Aerial/MeOH	45	90	NA	NA	NC
<i>Helminthotheca echiooides</i>	Aerial/DCM	49	100	NA	NA	NC
<i>Inula pseudolimonella</i>	Aerial/DCM	28	80	NA	NA	NC

**Table 2** continued

Plant	Plant part and extract	<i>L. donovani</i>		<i>P. falciparum</i>		Cytotoxicity
		IC <sub>50</sub> ( $\mu\text{g mL}^{-1}$ )	IC <sub>90</sub> ( $\mu\text{g mL}^{-1}$ )	IC <sub>50</sub> D6 ( $\mu\text{g mL}^{-1}$ )	IC <sub>50</sub> W2 ( $\mu\text{g mL}^{-1}$ )	
<i>Lavandula stoechas</i>	Aerial/MeOH	44	90	NA	NA	NC
<i>Leontodon tuberosus</i>	Whole/DCM	45	86	NA	NA	NC
<i>Luffa cylindrica</i>	Seeds/DCM	46	90	NA	NA	NC
<i>Luzia cretica</i>	Aerial/DCM	85	> 100	30	31	NC
<i>Nepeta melissifolia</i>	Aerial/DCM	45	92	NA	44	NC
<i>Onosma erecta</i> subsp. <i>erecta</i>	Aerial/DCM	48	98	NA	NA	NC
<i>Origanum dictamnus</i>	Aerial/DCM	9.2	18	46	40	NC
	Aerial/MeOH	36	89	NA	NA	NC
<i>Origanum microphyllum</i>	Aerial/DCM	8.8	17	46	30	NC
<i>Petromarula pinnata</i>	Aerial/DCM	100	> 100	NA	NA	NC
<i>Phlomis cretica</i>	Aerial/DCM	65	> 100	NA	NA	NC
<i>Sarcopoterium spinosum</i>	Aerial/DCM	44	86	NA	30	NC
	Aerial/MeOH	46	94	NA	NA	NC
<i>Sideritis syriaca</i> subsp. <i>syriaca</i>	Fl. stems/DCM	46	87	30	23	NC
<i>Stachys spinosa</i>	Aerial/DCM	53	> 100	25	22	NC
<i>Staelhelina petiolata</i>	Aerial/H <sub>2</sub> O	45	90	NA	NA	NC
<i>Tordylium apulum</i>	Rosette/DCM	17	73	38	29	NC
	Rosette/MeOH	54	> 100	NA	NA	NC
	Aerial/DCM	45	86	NA	NA	NC
<i>Verbascum arcturus</i>	Aerial/DCM	57	> 100	NA	NA	NC
OQ (Chloroquine)	NT	NT	0.015	0.135	NT	
ART (Artemisinin)	NT	NT	0.014	0.1	NT	
Pentamidine	1.3	5	NT	NT	NT	
Amphotericin	0.17	0.34	NT	NT	NT	

NA Not active, NT Not tested, NC Not cytotoxic

**Acknowledgments** Special thanks to Dr Th. Constantinidis from the Laboratory of Systematic Botany, Agricultural University of Athens, for verification of many plant specimens, and to USDA, ARS for financial support. The antiparasitic screening program at National Center for Natural Products Research is supported in part by United States Department of Agriculture (USDA) cooperative agreement no 58-6408-2-0009. The technical assistance of John Trott and Mahitha Orugnati with the biological assays is acknowledged.

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